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HIV Infection

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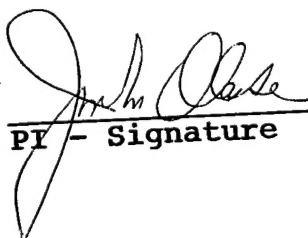
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## INTRODUCTION

In 1987, the risk of perinatal transmission of HIV from seropositive, pregnant women to their fetuses was estimated to be as high as 65%. Furthermore, it was projected that the steady increase in both IV drug abuse and heterosexual transmission of HIV in the United States would create a cohort of child bearing-age women infected with HIV who could potentially give birth to an estimated 10,000-20,000 cases of pediatric, perinatally acquired HIV infection by 1991.<sup>1</sup>

The State of New Jersey, at that time, ranked fourth in the nation in the number of AIDS cases reported to the Centers for Disease Control. The vast majority of HIV-infected patients resided in the Northern part of the state, inclusive of the Newark metropolitan area which was among the five metropolitan areas in the US with the highest concentration of AIDS cases. Since the majority of these patients were intravenous drug abusers (IVDAs) and/or their heterosexual contacts, there was a high concentration of high at-risk females and their offspring for experience and possible infection with HIV. Nationally, 58% of reported children with AIDS in 1987 were born to mothers who were IVDAs and/or sexual contacts of IVDAs. New Jersey ranked second in the nation with regard to the number of reported children with AIDS, with the Newark area being one of three metropolitan areas in the country with the highest concentration of infected infants.

During the early phase of the HIV epidemic, neither the spectrum and frequency of adverse effects of maternal HIV infection on the developing fetus (i.e. risk of fetal wastage, prematurity, small for date births, etc.) nor the possible adverse effects of pregnancy on the clinical course of an HIV infected woman were completely understood. Furthermore, it was not known whether maternal factors influenced the likelihood of perinatal transmission of HIV from mother to infant and which factors played a contributing role. If such determinants of transmission were to be identified, a model for understanding the mechanisms of vertical transmission of HIV could be developed that would provide insight into potential methods for prevention.

On September 30, 1988, in an effort to begin addressing these unknown questions, the Department of the Army/Institute of Research, through an interagency agreement with the National Institute of Allergy and Infectious Diseases (NIAID) funded a five year study (Contract No. DAMD17-88-C-8081) entitled Maternal Factors Influencing Perinatal Transmission of HIV-Infection. The contract was awarded to the Division of Allergy, Immunology and Infectious Diseases of the University of Medicine and Dentistry of New Jersey, New Jersey Medical School. The original contract period was from September 30, 1988 to December 29, 1993. Subsequent modifications of this contract (#P30008) revised the study period to September 30, 1988 to March 30, 1993 with a project completion date of September 30, 1994. This is the final report for this study.

## NARRATIVE

The details of the initial study protocol are outlined in Appendix I. It should be noted that over the course of the study, several changes were made to the protocol. The initial study design entailed a prospective evaluation of pregnant HIV infected women and their offspring (see Appendix II). Subjects were to be recruited into the study as early as possible in pregnancy with eligible patients being enrolled after written informed consent was obtained. Enrolled women were to be followed at defined intervals during pregnancy, at delivery, and postpartum. Infants born to enrolled mothers would be assessed for the presence or absence of HIV infection and if infected, the age at which their symptoms developed was to be documented. Infected infants would be transferred/referred to the Children's Hospital AIDS Program (CHAP) - the clinical arm of the Division of Allergy, Immunology and Infectious Diseases.

The Adult Obstetrical - Martland Clinic - at University Hospital/UMDNJ was designated as the primary source of the patients enrolled in this study. Historically, this clinic provided obstetrical care for the majority of the pregnant high-risk women in the Newark area and served as a referral center for the county drug rehabilitation program. A secondary source of patients for the study would include methadone maintenance clinics throughout the state that agreed to refer pregnant clients to the UMDNJ clinic; a recruitment effort by ex-addict health educators who worked the streets to identify and recruit pregnant IV drug abusers for the study was also developed.

At the enrollment visit, informed consent was to be obtained and baseline data relevant to the defined parameters being studied would be collected. These included:

1. a complete medical history, including previous pregnancies, specific symptoms related to HIV infection, and previous HIV testing;
2. demographic information (age, address, phone number, etc);
3. age of first drug use, frequency and type of drug use, sexual preference and practices, socioeconomic status, and family and social history;
4. a complete physical examination was performed on the mother with special attention paid to physical findings typical of HIV infection;
5. clinical staging of maternal HIV infection;
6. assessment of concomitant infections, specifically Herpes, CMV, chlamydia, toxoplasma, hepatitis, EBV, opportunistic infections;
7. immunological assessment including lymphocyte phenotyping and levels of total IgG, IgA, IgM

8. level of anti-HIV antibodies as measured by IFA and ELISA;
9. pattern of specific antibody response to HIV determinants as measured by Western Blot
10. presence of neutralizing antibodies for HIV in serum;
11. determination of the levels of maternal HIV antigen in serum.
12. presence of maternal HIV viremia;

**Year 1  
(1988-1989)**

For more than one year after the initial grant award notice, the project was directed **not** to enroll patients because a) of the need to make decisions about incorporating the project into other multicenter transmission studies and 2) the Army Surgeon General's IRB initial disapproval of the project over issues of "military significance" and "fetal research." Additionally, multiple personnel changes in both the Army and NIAID scientific staffs led to uncertainty about the scope and purpose of the project. Considerable work was done on a revised contract which eliminated (a) examination of fetal tissue, (b) enrollment of prisoners, and (c) enrollment of persons < 18 years of age (the latter was later permitted) to comply with the Army IRB.

While the above identified issues were under negotiation, all budgeted laboratory equipment items were reviewed by the Army contract officer and upon approval were purchased (Appendix III); new laboratory personnel were identified for the positions supported by this grant, the hiring process was completed and each individual was trained to perform the immunological and virologic laboratory parameters included in the protocol. Internal and external quality control programs were established and proficiency was validated for all laboratory studies prior to the initiation of patient enrollment. Systems and procedures for computerization of laboratory data including the development of an electronic tracking of repository specimens was also accomplished during the first year.

In February of 1989, representatives from the Army, new NIAID personnel and the Newark study team met to review the original proposal and to plan the implementation of the project. Since infants born to HIV + mothers in this project were to be followed in a study funded by the Centers for Disease Control (CDC), the scientific staff from this agency was often actively involved in deliberations. The outcome of this meeting resulted in a revised contract (scope of work), including specific accrual goals and a revised study design which was submitted to the collaborating agencies involved. All revisions were submitted to the New Jersey Medical School and Army Surgeon General's Institutional Review Boards (IRB). On June 14, 1989, the Army IRB reviewed the new proposal and the project was finally approved for enrollment of patients.



The five year study was revised in design to address the natural history of pregnancy among HIV-infected women as compared to at risk but HIV negative control women by monitoring these women during their pregnancy, labor and delivery. The women enrolled in this study were stratified according to whether or not they were actively using substances (i.e. IV drugs) that would influence complications and outcomes in their offspring.

The hypothesis to be tested in this study was that there were certain selected factors in HIV-infected pregnant women that influence the likelihood of transmission of HIV to their infants. Assuming that such determinants of transmission could be identified, it was felt that a five year study of this nature could provide a model for understanding the mechanisms of vertical transmission of HIV and provide insight into potential methods for prevention. Additionally, differences in pregnancy outcome might be identified between seropositive and seronegative women. Specific maternal historical and laboratory parameters were to be prospectively monitored to determine if they were predictive of HIV transmission from the pregnant women to their infants.

The primary objective of this project was to study the natural history of the pregnancies of HIV-infected women and HIV seronegative controls with a total initial study cohort projected to be 240 women stratified into the following groups:

- 1) HIV + women actively using drugs (n = 80)
- 2) HIV + women who have acquired HIV by heterosexual contact (n = 40)
- 3) HIV- women who are at risk for HIV infection due to drug use (n = 48)
- 4) HIV- women who are at risk of HIV infection due to heterosexual contact (n = 72)

The number of patients expected to be enrolled in each group reflected the known seroprevalence of HIV infection among pregnant women attending the prenatal clinics at University Hospital of the University of Medicine and Dentistry of New Jersey in 1987. Pregnant women who received prenatal care at UMDNJ's obstetrical clinic would be tested for HIV antibodies by ELISA and Western Blot. All women, ages 18 years or older, who were in groups outlined above and who were willing and able to provide written informed consent were recruited for enrollment. Women who were incarcerated were not enrolled in this study.

It was anticipated that study patients in Groups 1 and 3 would have fewer visits and might initially present in active labor; while patients in Groups 2 and 4 would be more compliant and have at least six prenatal visits. Based on results from anonymous seroprevalence (4-5%) studies of pregnant women in Newark in 1987, a policy was established by the Obstetrics Department of the New Jersey Medical School to routinely offer HIV counseling and testing to patients attending the prenatal clinic. Approximately 18% of the women who delivered at University



Hospital had no prenatal care and 60% of these were drug users. It was estimated that at least 40 seropositive pregnant women per year would be available for enrollment in this study.

HIV + mothers were invited to enroll their infants into other ongoing studies including our CDC study of HIV natural history of infants born to seropositive mothers and our NIH/NIAID clinical trials. Information regarding the HIV infection status of those infants was available to this study. Infants born to mothers who were HIV negative and those who did not wish to be part of the ongoing studies were followed in this study at 6, 15, and 18 months to determine outcome and HIV infection status (see Appendix II for a schedule of infant visits).

By the September 11, 1989 (last quarter of the first year of the study), active patient recruitment had begun. Study data collection forms were still undergoing modification.

### **Year 2 (1989-1990)**

In the first quarter of the second year, there was a turnover in nursing personnel and a new study coordinator was hired. The study team concentrated on patient recruitment. Throughout the year, the Study Coordinator provided orientation/in-service regarding HIV infection and the importance of HIV testing to various University Hospital units (obstetrical clinic, delivery room services and the pediatric specialty clinics) that were working closely with the study.

In October 1989, a site visit by the Human Use and Regulatory Affairs staff took place and problems surrounding the continued restrictions on enrollment of patients under 18 years of age and the restrictions regarding pathologic examination of products of conception were discussed. Because of significant number of patients who had enrolled in the study had progressed in their pregnancy to the time of delivery, a system was needed to assure the timely collection of placentas. Staff were recruited and trained in the collection and sampling of placental tissue and an on-call schedule for placenta collection was set up.

In February 1990, representatives from NIAID and the DOD site visited the project to discuss study progress, plans for systematic selection of control patients, data collection and computerization. Active recruitment up to this point had been limited to HIV positive patients due to the temporary reduction of the study staff and the need for balanced accrual. As a result of this meeting, it was decided that (1) two seronegative control mothers would be selected sequentially after an HIV positive patient was enrolled; and (2) the project would resubmit its request to allow recruitment of pregnant patients under the age of 18, since it was estimated by the study team that as many as one-third of potential participants

would be missed as a result of this age restriction. Another request was made to the Army's Human Subject Review Board in March 1990 to reconsider enrollment of patients under 18 years of age. The request was approved later that spring. Additionally, the project requested reconsideration of the restriction against pathological examination of products of conception, however, this was denied.

In April 1990, Dr. R. Hoff, the new Chief of Pediatric and Family Service in the Epidemiology Branch Chief at NIAID made a site visit the project. At this meeting, issues regarding placenta collection, finalization of urine toxicology procedures and uniformity of data collection with the Women and Infants Transmission Study (WITS) were discussed. As a result of the meeting, the protocol for toxicity was finalized and placental collection procedures were reviewed; additionally, following a review by Program Staff and the NJMS study team, data collection forms for the study were modified and subsequently submitted for approval. It was anticipated that final programming and data entry could occur in the fall of 1990. Up to this point, there had been excellent compliance with data collection, however, the ongoing modifications of data collection forms had hindered computerization. Furthermore, discussions were ongoing about the compatibility with the data collection forms of the Women and Infants Transmission Study (WITS).

By the end of the second year, thirty percent of the total five year patient accrual had been accomplished and over 1000 protocol mandated immunologic and retrovirologic tests had been performed. Ongoing modifications of the data collection forms, however, were hindering computerization of data.

### **Year 3 (1990-1991)**

By the third year of the study, HIV/AIDS had become the eighth leading cause of death for women in the United States, and the leading cause of death for African-American women in New Jersey and New York. Sixty-three percent of reported cases of women with AIDS were among those with a prior or present history of substance abuse.

Enrollment of patients continued with 56% of the total five-year accrual goal reached. Recruitment efforts were redirected to enrolling patients in those groups with the lowest accrual. Up to this point, the majority of study participants were being recruited from the routine prenatal and high-risk prenatal clinics at University Hospital. In the first quarter of the third year, the project established a screening and recruitment service for pregnant adolescents between the ages of 14 and 21 years, with the assistance of the Maternal and Infant Care Clinic (MIC) of the Department of Obstetrics. Study personnel conducted an inservice for the MIC staff; counseling and testing of this age-specific cohort began in the first quarter of

this grant period. These activities enhanced the project's ability to enroll patients.

In April 1991, following a joint site by the NIAID and CDC, approval was given to continue use of the study forms and questionnaires and to go forward with computer programming, data entry and analyses of data collected from this study. All laboratory data were already computerized.

By August of 1990, an HIV in Pregnancy Clinic, under the direction of Dr. Arlene Bardequez, was established at University Hospital. These changes in the prenatal clinics at University Hospital resulted in more centralization of obstetrical care for HIV infected pregnant women and better logistics of study management, especially in the areas of compliance with study visits and improved data collection. The recruitment of an OB fellow (Fetal/Maternal Medicine) also enhanced our ability to collect protocol directed clinical evaluations and specimens. The cohort of women and their infants enrolled in this study were initially named the Newark Perinatal HIV Transmission **Study** (PHS).

Linkages with the Women's HIV Clinic, under the direction of Dr. Patricia Kloser, a specialist in HIV in women and an investigator in this project continued so that follow-up of all seropositive patients after delivery was accomplished. At this time, 30% of the HIV positive women were being followed in this clinic.

During the third year, the project recruited a retrovirology Ph.D. This individual supported that development of new laboratory assays, including B<sub>2</sub> microglobulin, IgA testing and P24 hydrolysis and quantitative HIV cultures - all of which provided additional support for early diagnosis testing in this study cohort.

Compliance with study visits and data collection by this time has generally been 90% for patient visits and protocol mandated testing. Due to ongoing modifications of data collection forms, however, this material had not been fully computerized. In April 1991, following a joint site by the NIAID and CDC, approval was given to continue use of the study forms and questionnaires and to go forward with computer programming, data entry and analysis of data collected from this study. All laboratory data were already computerized.

#### **Year 4 - Year 5 (1991 - 1993)**

Treatment protocols for HIV-infected women were under development at this time, with the AIDS Clinical Trials Group at NIAID planning a study of zidovudine in pregnancy for the purpose of evaluating its efficacy in the prevention of perinatal transmission of HIV. Once these treatment protocols were finalized, it was anticipated that they would be made available to pregnant women enrolled in this natural history study, as the use of antiretroviral treatment would require that this

factor be considered in analysis of results from Study Groups 1 through 4. Methods for handling these variables were discussed with program scientific staff.

During the fourth year of the study, it was determined that in order to assess the influence of drug use during pregnancy on HIV infection and the outcome of infants born to these women as well as the influence of HIV infection itself, it would be essential to have controls for each of the study groups (1 & 2). The scope of work under this contract was amended to reflect a smaller study cohort (N = 163) stratified as follows:

- 1) HIV + women evidenced for active substance use (n = 45)\*
- 2) HIV + women who are negative for active substance (n = 29)\*
- 3) HIV- women who are at risk for HIV infection and who are negative for substance use (n = 49)\*\*
- 4) HIV- women who are at high risk of HIV infection and are positive for active substance use (n = 40)\*\*

\* HIV + women through 6 months postpartum visit

\*\* HIV- mothers followed through delivery visit

Effective as of March 9, 1992 (per Contract Modification #P30008) there were to be **no** new subjects enrolled in the study; and, those subjects already enrolled continued to be monitored in accordance with the protocol of the study. This early study closure was due to budgetary limitations by the funding agency (NIAID), data collection conflicts with other established NIAID-sponsored perinatal studies (WICS) and the lack of resources available from the Department of Army/Institute of Research to maintain this study. At study closure (6/30/93) the total number of pregnant women enrolled was 213 (89% of initial enrollment goal), and a total of 4880 protocol mandated immunological and retrovirological tests had been performed, including 408 serum saves and 546 cell saves (Appendix IV). The modified enrollment goals defined in Year 4 above for each group were over-enrolled (157%, 162%, 104% and 110%, respectively) (Appendix V.) The total number of evaluable cases (all maternal and infant follow up data available at time of report) was 164 (100% enrollment per modification P30008). The total number of evaluable infants was 146 (74 born to HIV-infected women and 72 born to HIV-negative mothers). Appendix VI lists the publications/abstracts resulting from this study to date.

## STUDY METHODS

This prospective study of HIV-positive and HIV-negative pregnant women receiving prenatal care at University Hospital, Newark was initiated in 1989. Its objective was to assess maternal factors associated with HIV-1 transmission, ascertain the immunological and virological profile of seropositive women during

pregnancy, evaluate pregnancy outcome of seropositive women, and evaluate the interaction of substance use and HIV disease on maternal and neonatal outcome. This final report for contract #DAMD17-88-C-8081 summarizes the comparison of sociodemographic characteristics, HIV-related manifestations, medical/obstetrical complications, and immediate neonatal outcome of the final evaluable 162 mother and 146 infant enrolled.<sup>2</sup>

### **Study Population:**

The study was conducted at University Hospital, Newark, a level 3 facility which provides prenatal care to women from inner-city Newark and surrounding communities. Patients were enrolled from two sources; a voluntary program established in the prenatal clinic in September 1989 offered HIV counselling and on site HIV testing to women receiving care in our Institution. During the counselling session patients deemed at risk of HIV-1 infection were also offered participation in the Newark, Perinatal HIV-1 transmission study (PHS). Factors judged to constitute high-risk for HIV-infection included: past or current history of substance use, partners of an HIV-1 infected male, partners of a bisexual or an IVDU male, sex workers, blood transfusion before 1985, or birth in a pattern II country. The research staff explained the study to each potential candidate, obtained the informed consent approved by the Institutional Human Research Committee, and scheduled study visits. This group of patients was enrolled prior to knowledge of their serostatus. In addition, HIV-positive women referred to our Institution for perinatal management, were also offered enrollment into the study. Patients were stratified into 4 different groups according to their HIV-serostatus and concomitant substance use as defined in study Year 4 modification.

### **Maternal HIV Diagnosis:**

HIV status was determined at entry using an Elisa assay (HIV-1 EIA: Abbott, Chicago, Illinois) and persistent positive samples were confirmed with Western blot (Dupont, Wilmington, Delaware). Interpretation of these assays was done according to the criteria of the Association of State Public Health Laboratory Directors/Centers for Disease Control and Prevention (ASTPHLD/CDCP) to classify patients as HIV-1 seropositive or seronegative. To ascertain any seroconversion after enrollment, HIV serostatus was reassessed in all seronegative participants during their follow-up visits.

### **Study Parameters:**

HIV-1 risk assessment, toxicology screen, clinical, and HIV-related laboratory evaluations were done at: (1) enrollment, (2)  $26 \pm 2$  weeks, (3)  $38 \pm 2$  weeks, (4) at delivery  $\pm 3$  days (5)  $6 \pm 2$  weeks postpartum, and (6) 6 months  $\pm 4$  weeks postpartum. Sociodemographic information, risk factor assessment, past medical

and obstetrical history, and information regarding past or current use of HIV-related therapies were collected using standardized forms. Substance use was assessed during confidential interview of participants at each visit, and confirmed by serum and urine toxicology assays. Urine was assayed for cocaine, marijuana, opiates, methadone, and benzodiazepines (Metpath laboratories, Teterboro, New Jersey). Each sample was screened by an immunoassay (EMIT) and confirmed by Gas Chromatography/Mass Spectrometry, except for benzodiazepines, which are performed utilizing HPLC with Photodiode Array detection. Serum samples were assayed for alcohol (Metpath laboratories, Teterboro, New Jersey) using the radiative energy attenuation technique (TDx REA Ethanol assay: Abbott, Chicago, Illinois).

Clinical evaluations consisted of a physical exam with emphasis on obstetrical/gynecological symptoms, and HIV-related manifestations. All participants had an obstetrical ultrasound done at baseline and during follow-up prenatal visits. Gestational age assignment was based on the best available obstetrical parameter at enrollment, namely the date of the last menstrual period (LMP) confirmed by sonogram, a physical exam prior to 20 weeks gestation or a sonographic estimate of the expected date of confinement (EDC) in patients with unknown LMP.

Genital herpes, (tissue culture, confirmed with immunofluorescence assay: Syva Company, Palo Alto, CA), chlamydia (tissue culture inoculation, confirmed by enzyme immunoassay: Meridian Diagnostics Inc., Cincinnati, Ohio) and urine cytomegalovirus (CMV) culture (tissue culture, confirmed with immunofluorescence assay: Syva Company, Palo Alto, CA) were done on the initial research visit. Hepatitis C serostatus was determined from saved serum specimens obtained at the initial visit. Samples were batched and assessed for Hepatitis C by measuring antibodies to the C100 protein (Anti C-100 assay: Abbott, Chicago, Illinois), a positive sample was confirmed by a neutralization assay or a second generation assay based on core-NS<sub>3</sub> peptide. Results of Hepatitis B serology (surface antigen, surface antibody, core antibody), syphilis serology (RPR confirmed by FTA), Neisseria gonorrhoea culture (Thayer Martin), Chlamydia trachomatis assay (Clamydiazyme or Microtract, Abbott, Chicago, Illinois) and cervical cytology performed as part of routine prenatal evaluations were also documented.

Medical records were reviewed to code clinical information regarding perinatal outcome including hospitalizations, medications and HIV related complications.

Blood samples were collected in tubes with no additives, and EDTA K<sub>3</sub>, or sodium heparin during study visits to assess immunological and virological parameters. All HIV-related assays, namely lymphocyte phenotyping, p 24 antigen, ICD p24, HIV culture, and HIV-PCR were done in an ACTG certified laboratory.



Lymphocyte subset analysis was done within 6 hours of collection using standard whole blood lysis methodology. Detailed description of the methodology to complete these assays has been previously published.<sup>3,4</sup> Beta<sub>2</sub> microglobulin ( $\beta_2$ M) was assessed after study completion on repository sera using an immunoassay by Abbott Laboratories, and a summary of the methodology used for this assay is detailed in the Appendix.

### **Outcome Measurements and Definitions:**

HIV-1 positive patients were assigned to a clinical category (asymptomatic, symptomatic or AIDS) based on the CDC 1993 Revised Classification for HIV-infection in adolescents and adults. A substance user was defined as a patient with history of substance use prior to or during the current pregnancy, and/or those with positive serum/urine toxicology during any study visit. STD's evaluations were classified as either positive or negative according to standard specifications for each diagnostic assay. Papanicolaou smears were classified as normal or abnormal (defined as atypia, dysplasia or malignancy). We defined hematological complications as (1) thrombocytopenia (platelet count  $\leq 100,000$  cells/ul) or (2) anemia (hemoglobin  $\leq 10$  g/dl or hematocrit  $\leq 30\%$  during pregnancy). Standard obstetrical definitions were used to describe perinatal outcome. Premature labor was defined as the onset of active labor prior to 37 weeks gestation, premature rupture of membranes (PROM) was the rupture of membranes prior to the onset of contractions, and oligohydramnios was defined as the largest pocket of fluid  $< 2$  cm in diameter in an obstetrical ultrasound. Neonatal birthweight, gender, and Apgar scores were coded from the medical records. Infants with 2 positive HIV-PCR (performed on repository specimen after study completion) or HIV-culture assays at two different visits, or with persistent HIV-antibody after 18 months of age were classified as HIV-infected. Those with 2 negative HIV-PCR or HIV-culture in which at least one of these evaluations was done after 6 months of age, or infants who seroreverted after 18 months of age were considered HIV-negative. Those unable or unwilling to complete the necessary evaluations for HIV-diagnosis after birth were considered lost to follow-up (17 infants).

### **Statistical Methods:**

Statistical analysis of this large cohort data set required provision of adequate computer facilities for data collection, quality checking, and analysis. The backbone of computing at the Institution is a Hewlett Packard 9000 Main frame Computer, capable of handling our data set's size and complexity. However, it was necessary to upgrade computer capabilities within the Division by providing local computers with terminal-emulator capacity, and the hardware and software needed for remote data entry. Also needed were suitable peripherals such as monitors, printers, modems and servers. Cabling was needed to allow data entry and access from laboratory and clinical locations, and to link these locations, the



mainframe and the terminal in the statistician's office. With all this in place, clinicians and laboratory personnel could send data to the main frame to be incorporated into the ongoing collection of data, and the data could be immediately accessed for data cleaning, revision, examination, and statistical analysis.

Statistical Analysis System (SAS) version 6.09 software (Cary, North Carolina) was used for statistical analysis. For the purpose of data analysis time points of study visits were defined as follows: (0) < 20 weeks gestation, (1) 24-28 weeks gestation, (2) 36-40 weeks gestation, (3) delivery, (4) 6 weeks postpartum and (5) 6 months postpartum.

Student's t-test was used to assess differences between infected and uninfected groups. If significant differences were observed between these groups Mantel-Haenszel  $X^2$  test or the two tailed Fischer's exact test was used for adjustment of stratified analysis. Analysis of variance was used to assess simultaneously the individual contributions of mother's HIV status, child HIV status, and mothers toxicology status as determinants of birthweight. Regression analysis was used to assess the impact of toxicology status on birthweight. Logistic regression was used to analyze the effect of toxicology on the HIV status of mother and child, and to assess the effects of HIV-status versus toxicology as determinants of anemia, thrombocytopenia, PROM and oligohydramnios. Univariate associations between these variables were assessed with chi-square analysis. A  $p \leq 0.05$  was considered significant.

## RESULTS

### **Sociodemographic and Clinical Characteristics of Patient Population:**

Seventy-four HIV-positive and ninety HIV-negative pregnant women were enrolled and evaluable at study completion as well as 146 infants born to these mothers. The average number of study visits per maternal patient was 3. Sociodemographic characteristics of the two groups are presented in Table I. Overall, ninety-six percent (155/162) of the women enrolled in the study belong to racial/ethnic minority groups. Sixty-five of the seropositive women (88%) were diagnosed during the current pregnancy through the counselling and testing program in the prenatal clinics. We did not ascertain factors associated with acceptance of HIV-testing during pregnancy, therefore the role of how ethnic/cultural attitudes and beliefs influence acceptability of HIV-testing and HIV-related care was not ascertained in this study. Consideration of these factors might be relevant to achieve the same degree of success when implementing counselling and testing programs in other communities.<sup>5-14</sup>

Nine patients (12%) received their HIV-diagnosis prior to pregnancy, and were referred to our Institution for HIV-related care. The most common HIV risk

factor among the participants (75%) was unprotected intercourse with a male partner at high risk for HIV-1 infection (heterosexual transmission) followed by intravenous drug use (16%). HIV-positive and HIV-negative women were comparable for racial/ethnic distribution, marital status, level of achieved education, and health insurance coverage. There was also no significant difference in racial/ethnic distribution among women with different HIV-disease stage. The mean age for the cohort was  $26 \pm 5$  years. However, HIV-positive women were older ( $p = 0.001$ ) and had higher parity ( $p = 0.002$ ) than seronegatives. A higher number of prior full term pregnancies among infected women [63/74:85.1% versus 54/90:60.0%,  $p = 0.001$ ] accounted for the difference in parity.

On the initial study visit the mean gestational age of study participants was  $20.3 \pm 7.6$ , nevertheless HIV-infected women enrolled significantly later to the study than their seronegative counterparts ( $p = 0.0001$ ). There were three spontaneous abortions, [HIV (+) 1/71 vs HIV (-) 2/88] in the cohort. In 1991 co-enrollment in perinatal protocols sponsored by the AIDS Clinical Trial Groups was available on site for seropositive women. During the study period only 5 women chose to enroll in the perinatal protocol ACTG 076, and only 1 of them was randomized to the active arm (zidovudine).

### **Substance Use:**

Forty-four percent (72/162) of the women in the cohort were substance users, and a significant association between substance use and HIV-status was noticed ( $p = 0.002$ ). Notwithstanding the high rate of substance use among infected subjects in general, only thirty-two percent (24/74) of the HIV-positive women had current or prior history of intravenous drug use. Crack/cocaine was the most commonly used recreational drug, followed by polydrug use and marijuana (tetrahydrocannabinol). Although many polydrug users acknowledged alcohol consumption during pregnancy and postpartum during study interviews only 0.81% (1/122) had toxicology validation of their intake.

### **HIV-Associated Findings:**

Fifty seropositive women were asymptomatic (50/72:69%), nine were symptomatic (9/72:13%) and thirteen (13/72:18%) had AIDS based on the 1993 CDCP classification. Two seropositive women with incomplete clinical information were not assigned to any HIV-disease stage. Forty-six percent (6/13) of the patients in the AIDS category met this definition based only on CD4 lymphocyte counts  $< 200$  cells /  $\text{mm}^3$  (stage A<sub>3</sub>). The AIDS defining diagnoses observed in the cohort were: *Pneumocystis carinii* pneumonia (N = 3), oroesophageal candidiasis (N = 3), miliary tuberculosis (N = 1), AIDS dementia (N = 2) and wasting syndrome (N = 1). Twenty-five patients used antiretroviral (zidovudine-ZDV) therapy during the study period, twelve received ZDV antepartum while 13 chose to initiate

therapy postpartum. Thirty-three percent (4/12) of the patients who received ZDV during the antepartum period had been on this medication for  $\geq 6$  months. Twelve patients received trimethoprim/sulfamethoxazole (TMP/SMX) during pregnancy for PCP prophylaxis or treatment, and one patient received rifamate (rifampin and isoniazid capsules) for treatment of tuberculosis. There were two maternal deaths in the HIV-infected group; one was secondary to *Pneumocystis carinii* pneumonia (PCP) and the other one was unrelated to HIV-infection (homicide). Both of these deaths occurred after delivery but prior to the 6 months evaluation.

The immunological characteristics of HIV-positive and HIV-negative women are detailed in Table II. As expected, a significant difference in absolute and percentage of CD4 and CD8 lymphocytes subsets was observed when comparing the two groups. Eight percent of the seropositive women had CD4 lymphocyte counts  $\leq 200$  cells/mm<sup>3</sup>. The mean absolute CD4 lymphocyte count of seropositive and seronegative women on the initial visit (earliest study visit) were  $513 \pm 292$  and  $994 \pm 298$  cell/mm<sup>3</sup> respectively ( $p = 0.0001$ ). Immunosuppression was more common among women with advanced disease. Mean absolute CD4 counts of asymptomatic, symptomatic and AIDS cases were  $592 \pm 244$ ,  $344 \pm 121$ , and  $128 \pm 81$  respectively.  $\beta_2$ M levels were also significantly higher among infected women than their uninfected counterparts.

The virological characteristics of HIV-1 infected women were evaluated at initial visit and at the time of delivery. The rates of positive HIV cultures ranged from 57.5-66.6%. There was a significant negative correlation ( $p = 0.001$ ) between absolute CD4 counts and positive HIV-1 culture. There was no intraassay variability in p 24 antigenemia when samples at entry and delivery were compared. However, a significant interassay variability was noticed between the standard p24 [Abbott] and the immune complex dissociation (ICD) assay [Coulter] ( $p = 0.00$ ). Higher rates of p 24 antigenemia were detected with the ICD p24 methodology. The rate of positive p 24 antigenemia at entry and delivery with the standard (Abbott) and dissociated (Coulter) methods were 3.3% versus 23%, and 1% versus 22% respectively. (Table III)

### **Sexually Transmitted Diseases:**

There was no significant difference in the rate of positive *N. gonorrhea*, asymptomatic CMV, or genital herpes shedding from the genital tract between the HIV-positive and the HIV-negative groups. Irrespective of the assay used to assess for chlamydia trachomatis infection (culture or immunofluorescence) no significant difference in infection rates was noticed within each group. Prior exposure to Hepatitis B infection (defined as a negative surface antigen with positive surface antibody) was common among women enrolled in the study (23/61: 38%), however no significant difference was observed between infected and uninfected women. (12/22: 54% versus 11/39: 28%  $p = 0.13$ ). A significant difference in

positive hepatitis B antigenemia was observed between the study groups (15/44: 34.0% versus 0/82: 0%,  $p=0.0001$ ). This difference was unrelated to the use of recreational drugs in the study population ( $p=0.14$ ). Forty-one percent of the participants had evidence of prior exposure to Hepatitis C infection (defined as a positive antibody to C<sub>100</sub> protein). HIV-1 infected women were more likely to have had Hepatitis C infection than their uninfected counterparts ( $p=0.018$ ). The rate of positive syphilis serology was also significantly higher in HIV-infected women ( $p=0.001$ ). Among HIV-positive women a positive syphilis serology test was more likely to be found in substance users ( $p=0.0002$ ). (Table IV)

### **Obstetrical and Gynecological Complications:**

We compared the rate of perinatal complications between the study groups. No significant difference was noted for gestational diabetes, pregnancy induced hypertension, malpresentation, fetal distress or meconium stain. There was no significant difference between the groups for conditions generally associated with an infectious etiology such as preterm labor, urinary tract infection, and endometritis.

Also remarkable was the lack of significant difference with conditions generally associated with substance use such as wound dehiscence and abruptio placenta. The only obstetrical and gynecological complications in which significant differences were observed between the groups were oligohydramnios ( $p=0.014$ ), PROM ( $p=0.006$ ), abnormal pap smears ( $p=0.0001$ ) and postpartum bleeding ( $p=0.01$ ). HIV-positive women were also more likely ( $p=0.006$ ) to have symptoms associated with clinical evidence of genital candida infections. [Table V] Anemia and thrombocytopenia were the only medical diagnoses seen more frequently among infected subjects ( $p=0.0002$  and  $0.006$ , respectively) [Table VII]

There was no univariate association between oligohydramnios ( $p=0.45$ ), PROM ( $0.46$ ), anemia ( $p=0.25$ ) or thrombocytopenia ( $p=0.596$ ) and substance use, suggesting that these complications were related to the HIV-infection status rather than substance use as a confounder. Logistic regression equation which sequentially incorporated HIV-status and substance use, and as dependable variable any one of these medical complications (oligohydramnios, PROM and anemia) confirmed that only HIV-infection was close to significance ( $p=0.058$ ). From the logistic regression the odds/ratio for PROM, oligohydramnios, and anemia in the HIV-infected women versus their seronegative counterpart was 11.1 (95% CI 1.36-91.2), 4.67 (95% CI 1.24-17.7), and 4.5 (95% CI 1.87-10.9) respectively. Substance use was already in the regression equation but not significant, and so a correlation was unnecessary. Thrombocytopenia as defined in our study was only documented in HIV-infected women. The association between the degree of immunosuppression (defined as CD4 lymphocyte count  $>$  or  $<$  500 cells/mm<sup>3</sup>) and

the occurrence of these complications was also evaluated. Only thrombocytopenia ( $p=0.0002$ ) and abnormal papanicolaou smears ( $p=0.037$ ) were significantly associated with CD4 lymphocyte counts  $<500$  cells/mm<sup>3</sup>.

### Infant:

Table VII illustrates the immediate neonatal outcome of all infants in the cohort. There was only one infant with a minor congenital anomaly [HIV(-) group] in the cohort. There were no stillbirths, however 2 neonatal deaths occurred among infants born to seropositive women. The HIV-infection status of these infants at the time of death was indeterminate. The cause of death were Group B streptococcus sepsis 8 hours after birth, and Sudden Infant Death Syndrome (SIDS) at 4 months of age. There were no difference in gestational age at birth, prematurity, percentage of male infants, mode of delivery or Apgar score at 1 minute between the groups. Infants born to seropositive women were more likely to have an Apgar score at 5 minute  $<7$  ( $p=0.02$ ). However, the mean gestational age for these infants was  $29\pm8$ , and sixty percent of them (3/5) were born to women who used illicit drugs. The low birthweight rate in the cohort (139/1000) was higher than the national average (75/1000), but no significant differences were observed between the groups. Only two of these infants had birthweights below the 10th percentile for their gestational age. There was only one VLBW infant born to an HIV-negative women.

There was no statistical difference in mean birthweight between infants born to HIV-infected and uninfected women ( $p=0.12$ ) [Table VII]. As expected, there was a significant correlation between birthweight and gestational age ( $p=0.0001$ ). There was also a significant correlation between birthweight and infant gender ( $p=0.0001$ ), with higher birthweights among male than female infants. There was no correlation between birthweight and maternal race/ethnicity ( $p=0.71$ ). Comparison of birthweights in HIV-infected and HIV-uninfected infants revealed no significant difference between these groups [ $2894\pm459$  vs  $3030\pm568$ ,  $p=0.1248$ ]. A significant correlation ( $p=0.004$ ) between birthweight and maternal substance use was seen. The birthweight of infants born to mother with or without substance use was  $2926\pm497$  and  $3223\pm586$ , respectively. In a linear regression model substance use (+ toxicology) was found to be a significant determinant of birthweight ( $p=0.0045$ ); maternal HIV-status ( $p=0.52$ ) or infant HIV status was not ( $p=0.54$ ). The mean birthweight for every combination: Maternal HIV status, infant status (infected or uninfected) and substance use (positive or negative toxicology) is illustrated in Table VIII.

Eight infants were classified as HIV-infected, and three neonates were lost to follow-up. Overall, there were 6 infants with unknown HIV-1 serostatus who were potentially at risk of perinatal transmission [1 abortion, 2 neonatal deaths, and 3 lost to follow up]. From this information we can infer that in the best case

scenario (if all infants with unknown serostatus were HIV-negative) the perinatal HIV-1 transmission rate in the cohort would be 11% (8/74), and in the worse case scenario (if all infants with unknown serostatus were HIV-positive) the transmission rate would be 19% (14/74). Nevertheless, the HIV-1 perinatal transmission rate observed for infants followed in our cohort was 12% (8/66). The mean absolute CD4 lymphocyte count at delivery for mothers with infected infants was  $433 \pm 294$ , and seventy five percent (6/8) of these women had absolute CD4 lymphocyte counts  $< 500$  cells/mm<sup>3</sup>. Two of these women received oral ZDV only during the antepartum period for maternal indications. An additional participant randomized to the active arm of the ACTG 076 protocol [zidovudine antepartum and intrapartum to the mother and to the neonate for 6 weeks] had an HIV-negative infant.

Taking advantage of the study repository with 408 serum, 546 cell and 88 viral isolates, additional studies are in the planning stages including analysis of maternal/fetal immunoglobulin transplacental transport and a study in progress with Drs. Merlin Robb and Doug Mayers in which infant and maternal specimens will be used to study the incidence of antiretroviral resistance.

## CONCLUSIONS

This 5 Year study showed that HIV-1 infection can adversely impact pregnancy outcome and women's health, independent of substance use. On the other hand, substance use was the most important factor influencing immediate neonatal outcome. The increased rate of hematological complications observed in HIV-infected pregnant women, concur with prior observations of anemia and thrombocytopenia in other cohorts.<sup>13</sup> Indeed, anemia has been reported more frequently among infected females with advanced HIV-disease, irrespective of the use of antiretroviral therapy [Nahlen B. et al Anemia among HIV-infected adults in the US International Congress on AIDS /STD's, Berlin 1993]. In the past, these hematological complications have been associated with adverse perinatal outcome such as intrauterine growth retardation, stillbirths, and postpartum bleeding. Therefore, institution of therapy during the prenatal period could benefit both the pregnant women and her unborn child. Initial data from this study provided part of the information base to justify the development of ACTG 076.

Cytologic screening of the cervix (papanicolaou smears) provides a rapid, cost effective tool for the detection of cervical neoplasia. It allows us to prioritize diagnostic work-up and treatment of malignant and premalignant lesions during pregnancy and postpartum. Higher rates of abnormal cervical cytology, cervical dysplasia and neoplasia has been previously reported in HIV-infected women.<sup>5</sup> In most instances the subjects had advanced HIV-disease or higher degree of immunosuppression (low CD4 lymphocyte counts). Some investigators have also reported higher failure rates to conventional ablative therapy in HIV-infected



women.<sup>6</sup> The high rate of abnormal papanicolaou smears in the HIV-infected pregnant women when compared to their seronegative counterparts [17/56:30% versus 1/58:1.7%] stresses the importance of this screening tool during the prenatal care period. Also remarkable, was our observation that only 33% of the women with abnormal pap smears had CD4 lymphocyte counts  $< 500/\text{mm}^3$  which emphasizes the importance of close surveillance for cervical neoplasia among all HIV infected women. Early diagnosis, treatment and long term surveillance of cervical dysplasia could decrease mortality and long term morbidity of infected women.

Syphilis and hepatotropic infections were the only sexually transmitted diseases occurring more frequently among HIV-infected pregnant women in our cohort. These findings are in agreement with prior reports in pregnant and non-pregnant subjects.<sup>7,8,13</sup> The higher rate of Hepatitis B virus antigenemia in seropositive pregnant women also stresses the relevance of HBV screening during pregnancy to decrease infectious morbidity to the newborn infant, and the importance of immunizing HIV-infected women with no prior evidence of HBV infection. The rate of chlamydia trachomatis infection in our study population was comparable to rates observed in non-pregnant inner city women attending sexually transmitted diseases clinics. Nonetheless, we found no significant difference in the prevalence of Chlamydia trachomatis infection when we compared a rapid diagnostic assay to the traditional cell culture. Similar to the findings of Alger and collaborators the prevalence of chlamydia and gonorrhea infections were similar in infected and uninfected pregnant women.<sup>13</sup> The shedding of herpes from the genital tract was rare in study participants, however our restriction to one evaluation per patient could explain these findings. The prevalence of CMV infection in this cohort was 2%, which is comparable to the rates reported among women with an AIDS diagnosis, but lower than those expected in populations of low socioeconomic income.<sup>9</sup> Our low CMV prevalence rates could also be explained by the site where the specimen was collected, or the method used to assess for CMV infection and thus need further analysis in larger cohorts of pregnant women. Although vulvovaginal candidiasis is a common complication of pregnancy, seropositive women were more likely to have symptomatic candida infections.

An increased controversy has been elicited over the last decade regarding the effect of HIV-1 infection in perinatal outcome. Although some authors suggest that HIV-infection does not adversely impact obstetrical outcome,<sup>10</sup> others have shown an increased association of HIV-infection with the presence of sexually transmitted diseases, infectious morbidity during pregnancy and postpartum, low birthweight infants and increased immunosuppression.<sup>7,11,13,21</sup> The discordant findings in these studies could be the result of confounding variables such as delayed access to the health care system, substance use among study subjects, stage of HIV-disease or degree of immunosuppression.



Analogous to the report of Gloeb and collaborators<sup>13</sup> premature rupture of membranes was more frequently seen in HIV-infected subjects, and this association remained even after controlling for substance use. We also observed an increased rate of oligohydramnios, and postpartum bleeding among HIV-infected women unrelated to use of illicit drugs, antiretroviral therapy or thrombocytopenia. The pathogenesis of oligohydramnios, PROM, and postpartum bleeding in the HIV-infected pregnant woman is currently unknown. In our study, seropositive women were not at greater risk of other common medical, obstetrical or gynecologic complications. An interesting observation was that the degree of immunosuppression was only significantly associated with the occurrence of thrombocytopenia and abnormal papanicolaou smears. The role of antiretroviral therapy in decreasing adverse perinatal outcome should be assessed in a larger cohort of HIV-infected women with advanced disease.

$\beta_2$  microglobulin, a non specific marker for immune activation which has been associated with disease progression in cohorts of homosexual men, was elevated in the HIV-infected pregnant women.<sup>3</sup> This surrogate marker could be used to predict disease progression or to monitor response to HIV-related treatment. Nonetheless, the correlation of  $\beta_2$ M with lymphocyte subsets, clinical disease, and the normal levels for pregnancy, need to be evaluated further. Similar to prior reports in pregnant and non-pregnant adults HIV-infected women had decreased CD4 counts when compared to their seronegative counterparts, which was inversely correlated with their HIV-disease stage.<sup>11,12,13</sup> Eight percent of the women had CD4 lymphocyte counts  $<200/\text{mm}^3$ , which illustrates the degree of immunosuppression that can be found among pregnant women.

Over sixty-percent of the patients had positive viral culture, and more than twenty percent of them had a positive p 24 antigen with the ICD assay demonstrating the continuous viral replication characteristic of HIV disease. Our study also confirms prior reports by other investigators regarding the increased sensitivity of the ICD p 24 assay in monitoring p 24 antigen fluctuation in infected subjects. Thus the higher rate of p 24 antigenemia detected in our cohort of pregnant minority women compared to prior reports in non-pregnant IVDU's could be related to our choice of methodology for p 24 antigen evaluation.<sup>15</sup> Only 1-3 % of the pregnant women in our cohort were p 24 antigen positive with the standard Abbott assay versus 22-23% when the ICD p 24 assay was used. Our observations of viral replication during pregnancy and viral antiretroviral resistance should be confirmed in larger cohorts since this information is important in facilitating accurate monitoring of disease progression and response to treatment during pregnancy. Based on this data, a study of antiretroviral resistance is being conducted on stored specimens with Dr. Doug Myers at the US Army Research and Development Command.

Similar to observations by other investigators, we found that HIV-infected

women were more likely to be older, have higher parity and seek prenatal care later than seronegative women from the same clinic.<sup>13,14</sup> This emphasizes the importance of preconception HIV-counseling and testing for women of reproductive age, and the need to educate women to seek prenatal care early if they truly desire to exert their reproductive choices. It also demonstrates the need for non-invasive procedures in order to minimize the risk for perinatal transmission in HIV-infected women with advanced maternal age. The similarities in the sociodemographic profile of infected and uninfected pregnant women underscore the importance of HIV-counseling and testing of sexually active women in order to identify asymptomatic subjects early in the disease process.

Sexual contact with a high risk male was the most common exposure risk for HIV-infection among the reproductive age women who participated in our study. This observation concurred with national and international trends which document heterosexual transmission as the leading exposure category for this population. Fifty-eight percent of the HIV-infected women were substance users, but only thirty-four percent of them had current or prior history of IVDU. This information emphasizes the important interaction of the HIV and substance use epidemics in reproductive age women living in inner-city communities<sup>8,10</sup> Documentation of substance use was more precise in our study since we combined patient history and toxicology screening during pregnancy to ascertain substance use among participants. This approach was utilized to avoid the under reporting of substance use previously documented in ambulatory health care settings. Efforts to decrease HIV-infection among women must utilize innovative approaches that integrate substance use and HIV-prevention strategies.

The majority of the HIV-infected women in our cohort were clinically asymptomatic (69%). However a significant number met the 1993 CDC criteria for initiation of antiviral therapy (25/72: 35%) or PCP prophylaxis (6/72: 8%). The increased morbidity and mortality of HIV-infected women has been related to delays in diagnosis and treatment. The death rate among immunosuppressed women ( $CD4 < 200/mm^3$ ) was lower than prior reports which probably reflects our aggressive initiation of PCP prophylaxis and/or antiretroviral therapy in this population. HIV-related therapy should not be deferred to the postpartum period in immunosuppressed women if we are to avoid the poor pregnancy outcomes reported in the mid 80's.<sup>9,17,18,20</sup> Similar prior reports in pregnant and non-pregnant women, PCP and oroesophageal candidiasis were the most common AIDS defining diagnosis.<sup>9,12,21</sup> This pattern of AIDS defining illness is different from that seen among IVDU's males and homosexual subjects and highlights the potential role of gender, age or race in the clinical presentation of HIV-disease.<sup>9</sup> In spite of the high rate of substance use in the population most of these women were not intravenous drug users, which can explain the difference in clinical manifestations of HIV-disease in these women when compared to male cohorts.

In spite of the increased degree of immunosuppression among many HIV-infected women enrolled in this cohort we had a low perinatal transmission rate (12%) when compared to national statistics (13-33%).<sup>22</sup> We cannot explain at the present time the reason for this finding. However, it has been suggested that obstetrical practices such as the use of invasive procedures or the mode of delivery; viral characteristics such as phenotype and virulence; and host characteristics such as the presence of neutralizing antibodies or the level of cytotoxic CD8 activity could influence perinatal transmission. These factors should be prospectively evaluated.

When controlling for gender and gestational age the birthweight of infants born to infected and uninfected women was similar, which is consistent with recent reports of prospective cohorts in the US. The strong association of low birthweight and low Apgar scores at 5 minutes, with use of illicit drugs, emphasizes the relevance of providing access to substance use treatment programs, as an integral part of the prenatal care to the HIV-infected pregnant women. It is also noteworthy to recognize that contrary to observations in underdeveloped countries, HIV-infection has minimal impact on the immediate neonatal outcome of an infant born to an HIV-infected woman. The potential long term sequelae of perinatal exposure to illicit drugs in HIV-infected infants merits further evaluation.

In summary, our study identified obstetrical complications amenable to therapeutic intervention. Correction of anemia and thrombocytopenia, and treatment of abnormal pap smears, can decrease long term morbidity and improve the quality of life of infected women. Our observations underscore the importance of substance use as an important cofactor in explaining the adverse perinatal outcome previously ascribed to HIV-infection. We face new challenges in understanding the pathophysiologic mechanisms for some of our observations in the woman with HIV infection. This study demonstrates the independent role of HIV-infection and substance use in the prevalence of such complications, and correlates their frequency to the immune status of these women. Finally, we confirmed recent observations of changes in sociodemographic profile of HIV-infected women which should lead to policy modification regarding prevention strategies, access to HIV-related therapies for the childbearing age women, in the context of prenatal care.

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Table I: Sociodemographic Characteristics

	HIV positive N=74	HIV negative N=90	p
Age (years)	27.8 $\pm$ 5.4	25.1 $\pm$ 5.0	0.001
Gravity	3.8 $\pm$ 2.1	3.4 $\pm$ 1.8	0.15
Parity*	1.9 $\pm$ 1.5	1.2 $\pm$ 1.3	0.002
Race/ethnicity			0.17
African-American	57 (79.1%)	78 (86.6%)	
Hispanic	6 (8.3%)	9 (10.0%)	
Haitian	4 (5.5%)	1 (1.1%)	
White	5 (6.9%)	2 (2.2%)	
Married	16/74 (21.6%)	9/86 (10.4%)	0.08
Education**	32/68 (47.0%)	45/81 (55.5%)	0.38
Health care coverage			0.79
Private Insurance	14/68 (20.5%)	19/80 (23.7%)	
Medicaid/none	54/68 (79.4%)	61/80 (76.2%)	
Risk Factor			0.000007
IVDU	24 (32.4%)	3 (3.3%)	
Sexual contact	43 (58.1%)	80 (88.8%)	
Blood transfusion	1 (1.3%)	2 (2.2%)	
Unknown	6 (8.1%)	5 (5.5%)	
Substance use***	42/72 (58.3%)	30/90 (33.3%)	0.002
Gestational age at entry	24.4 $\pm$ 7.2	15.5 $\pm$ 4.7	0.0001

\*Number of full term and preterm deliveries

\*\*Completed 12 years or more of education

\*\*\*Defined by positive history or toxicology

**Table II: Immunological Characteristics (Mean  $\pm$  SD) Of HIV-Positive  
And HIV-Negative Pregnant Women On Initial Visit\* And At Delivery\*\***

	HIV-positive		HIV-negative		p*	p**
	Visit (O)	Delivery	Visit (O)	Delivery		
CD4%	29.4 $\pm$ 11.7 (N = 40)	29.8 $\pm$ 13.2 (N = 47)	50.3 $\pm$ 7.3 (N = 48)	50.4 $\pm$ 8.1 (N = 57)	0.0001	0.0001
CD4	485 $\pm$ 274 (N = 40)	589 $\pm$ 416 (N = 47)	1027 $\pm$ 327 (N = 48)	1148 $\pm$ 458 (N = 57)	0.00001	0.0001
absolute	(N = 40)	(N = 47)	(N = 48)	(N = 57)		
CD8%	54.6 $\pm$ 11.7 (N = 40)	51.1 $\pm$ 11.6 (N = 47)	33.6 $\pm$ 7.0 (N = 48)	33.7 $\pm$ 7.1 (N = 57)	0.0001	0.0001
CD8	893 $\pm$ 451 (N = 40)	888 $\pm$ 345 (N = 47)	696 $\pm$ 262 (N = 48)	749 $\pm$ 249 (N = 57)	0.0175	0.0240
absolute	(N = 40)	(N = 47)	(N = 48)	(N = 57)		
$\beta_2$ M	1.79 $\pm$ 1.0 (N = 29)	1.93 $\pm$ 1.1 (N = 23)	0.99 $\pm$ 0.37 (N = 39)	1.33 $\pm$ 0.53 (N = 22)	0.0001	0.0056

$\beta_2$ M: Beta 2 microglobulin

\* Visit (O): Defined as visit prior to 20 weeks gestation

\*\* Delivery: Immediately postpartum  $\pm$  3 days



**Table III. Virological Characteristics of HIV+ Pregnant Women on Initial Visit and at Delivery\*\***

HIV-Positive			
	Visit (0) *	Delivery**	P
HIV-1 culture	28/42 (66.6%)	19/33 (57.5%)	0.84
p24 Antigen	3/91 (3.3%)	1/80 (1.3%)	0.360
ICD p24 antigen	17/73 (23.3%)	15/67 (22.4%)	0.94

HIV-1 culture PBMC culture (% of positive cases)

p24 antigen standard HIV-1 p24 antigen assay [Abbott] (% of positive cases)

ICD p24 immune complex dissociation assay [Coulter] (% of positive cases)

\* Visit (0) defined as visit prior to 20 weeks gestation

\*\* Delivery immediately postpartum  $\pm$  3 days

**Table IV: Sexually Transmitted Diseases In HIV-Positive  
And HIV-Negative Pregnant Women**

	<b>HIV-positive</b>	<b>HIV-negative</b>	<b>p</b>
Gonorrhea	5/55 (9.0%)	3/81 (3.7%)	0.194
CMV	1/42 (2.3%)	0/35 (0%)	0.55
Herpes	2/37 (5.4%)	0/35 (0%)	0.32
Chlamydia culture	5/61 (8.19%)	6/64 (9.3%)	0.54
Chlamydiazyme	8/44 (18.1%)	8/60 (13.3%)	0.37
Hepatitis B ag	15/44 (34.0%)	0/82 (0%)	0.0001
Hepatitis B ab	12/22 (54.5%)	11/39 (28.2%)	0.13
Hepatitis C	35/69 (50.7%)	11/42 (26.1%)	0.018
Syphilis	18/54 (33.3%)	6/79 (7.5%)	0.001

**Table V: Obstetrical And Gynecological Complications In  
HIV-Positive And HIV-Negative Pregnant Women**

	<b>HIV-positive</b>	<b>HIV-negative</b>	<b>p</b>
Abruptio placenta	2/70 (2.8%)	0/90 (0%)	0.19
Oligohydramnios	10/62 (16.1%)	3/87 (3.4%)	0.014
PROM*	8/64 (12.5%)	1/89 (1.1%)	0.006
Preterm labor	8/64 (12.5%)	7/83 (8.4%)	0.322
PIH**	5/67 (7.4%)	2/88 (2.2%)	0.140
Gestational Diabetes	2/70 (2.8%)	4/86 (4.6%)	0.451
UTI	2/70 (2.8%)	6/84 (7.1%)	0.223
Malpresentation (breech)	1/71 (1.4%)	6/84 (7.1%)	0.102
Meconium stain	14/58 (24%)	15/75 (20%)	0.39
Fetal distress	5/67 (7.4%)	4/86 (4.6%)	0.36
Postpartum bleeding	5/67 (7.4%)	0/90 (0%)	0.01
Blood transfusion	3/69 (4.3%)	0/90 (0%)	0.08
Endometritis	3/69 (4.3%)	5/85 (5.8%)	0.489
Wound dehiscence	3/69 (4.3%)	1/89 (1.1%)	0.231
Genital Candida (symptomatic)	6/66 (9.0%)	0/90 (0%)	0.006
Abnormal pap-smear*	17/56 (30.3%)	1/58 (1.72%)	0.0001

\* Premature rupture of membranes

\*\* Pregnancy Induced Hypertension

\*\*\* Abnormal pap-smear: Defined as atypia, dysplasia or malignancy

**Table VI: Medical Complications In HIV-Positive And HIV-Negative Pregnant Women**

	<b>HIV-positive</b>	<b>HIV-negative</b>	<b>p</b>
Anemia** ( < 10g/dl)	23/49 (46.9%)	8/82 (9.7%)	0.0002
Thrombocytopenia*** ( $\leq$ 100,000 cells/ $\mu$ l)	6/66 (9%)	0/90 (0%)	0.006
Dermatitis	3/69 (4.3%)	0/90 (0%)	0.08
Asthma	3/69 (4.3%)	4/86 (4.6%)	0.622
(+) PPD	5/67 (7.4%)	5/85 (5.8%)	0.481

\* Defined as patients with either thrombocytopenia or anemia.

\*\* Defined as a hemoglobin  $\leq$  10 g/dl or hematocrit  $\leq$  30% during pregnancy.

\*\*\* Defined as platelet count  $\leq$  100,000 cells/ul

**Table VII: Neonatal Outcome Of Infants Born To HIV-Positive And  
HIV-Negative Pregnant Women**

	<b>HIV-positive</b>	<b>HIV-negative</b>	<b>p</b>
Gestational age	37.8 $\pm$ 2.6 (N = 68)	38.1 $\pm$ 2.5 (N = 78)	0.99
Birthweight	3008 $\pm$ 546 g(N = 67)	3160 $\pm$ 574 (N = 64)	0.12
Abortions	1/71 (1.4%)	2/88 (2.2%)	0.58
Prematurity <sup>*</sup>	8/27 (29.6%)	13/57 (22.8%)	0.50
Male gender	35/67 (52.2%)	31/73 (42.4%)	0.32
Apgar score 1 min (< 7)	15/49 (30.6%)	11/54 (20.3%)	0.24
Apgar score 5 min (< 7)	5/59 (8.4%)	0/66 (0%)	0.02
LBW <sup>**</sup>	10/57 (17.5%)	6/58 (10.3%)	0.24
VLWB <sup>***</sup>	0/67 (0%)	1/63 (1.5%)	0.488
LGA <sup>****</sup>	2/65 (3.0%)	4/60 (6.6%)	0.318
Operative delivery	18/68 (26.4%)	9/73 (12.3%)	0.05
1 <sup>o</sup> C/S	10	4	
Repeat C/S	8	5	

<sup>\*</sup> Prematurity: GA at birth <37 weeks

<sup>\*\*</sup> LBW:Low birth weight, birthweight 2,500 g

<sup>\*\*\*</sup> VLWB:Very low birth weight, birthweight <1500 g

<sup>\*\*\*\*</sup> LGA: Large for gestational age, birthweight >4000 g C/S: Cesarean section Vaginal:

defined as spontaneous delivery or a delivery assisted by forceps or vacuum

**Table VIII: Birthweight (Mean $\pm$ SD) Of Infants By:  
Maternal HIV-Status And Substance Use (Positive Or Negative Toxicology)  
And HIV-Status Of The Infant (Infected Or Uninfected)**

<b>Status</b>	<b>N</b>	<b>Birthweight</b>
HIV( +) Child HIV( +) Tox(-)	3	2923 $\pm$ 539
HIV( +) Child HIV( +) Tox( +)	5	2877 $\pm$ 472
HIV( +) Child (not +) Tox(-)	23	3204 $\pm$ 564
HIV( +) Child (not +) Tox( +)	36	2909 $\pm$ 533
HIV(-) Child (not +) Tox(-)	41	3270 $\pm$ 607
HIV(-) Child (not +) Tox( +)	23	2963 $\pm$ 461

APPENDIX I  
Maternal Evaluation

	First Visit	24 ± 2 Weeks	36 ± 2 Weeks	Delivery ± 48 hrs.	6 Wk Post Partum	6 Months Post Partum
<u>History and Physical</u>	x	x	x	x	x	x
<u>Vaginal Exam</u>	x		x	x		
C/S HSV	x		x	x		
Chlamydia	x		x	x		
G.C.	x			x		
<u>Urine</u>	x	x	x	x	x	x
Drug Screen	x		x	x		
CMV Culture	x		x	x		
<u>Blood/Lab</u>	x			x		
CBC/diff	x			x		
VDRL	x			x		
Rubella	x			x		
SMA 18	x			x		
Toxotiter	x			x		
Hepatitis B	x			x		
EBV Titer	x	x		x		
Alcohol level	x	x	x	x	x	x
<u>Immunology/ Retrovirology</u>	x					
IgG, A, M	x			x		
T&B/mitogens	x	x	x	x	x	x
EIA/ELISA+Ag	x	x	x	x	x	x
Std ELISA	x					
Western Blot	x	x	x	x	x	x
Neutralizing Ab	x	x	x	x		
HTLV-Ab	x			x	x	x
HIV Culture-(blood)	x	x	x	x		
<u>Serum Save</u>	x	x	x	x	x	x
<u>Cell Save</u>	x	x	x	x	x	x
<u>Ultrasound every two months</u>						
<u>Amniocentesis as clinically indicated</u>						



**APPENDIX II  
INFANT EVALUATION**

	<b>6 Months</b>	<b>15 Months</b>	<b>18 Months</b>
<b>Clinical Evaluation</b>	<b>x</b>	<b>x</b>	<b>x</b>
<b>HIV ELISA</b>	<b>x</b>	<b>x</b>	<b>x</b>
<b>Qlg G, A, M</b>	<b>x</b>	<b>x</b>	<b>x</b>

These data were available from the CDC sponsored study.

**APPENDIX III**  
**Laboratory Statistics**  
**September 30, 1988 - June 30, 1993**

Test	Grant Year				
	9/89-9/90	10/90-9/91	10/91-9/92	10/92-6/93	Study Totals
L y m p h o c y t e Phenotyping	99	208	202	12	512
Placenta Collection	15	30	28	1	74
Plasma Culture	0	67	106	5	178
HIV Culture	139	144	125	2	410
HIV Antibody (ELISA & WB)	267	478	390	9	1144
Serum Save	161	140	107	0	408
Cell Save	149	196	189	12	546
Urine Save	77	155	135	2	369
End Point Culture	0	68	119	5	192
HIV p24 Antigen	170	186	173	11	540
HTLV I/II	173	180	159	1	513

**APPENDIX IV**  
**Enrollment Statistics**  
**Period: September 30, 1988 through June 30, 1993**

Grant Year	1988-1989 <sup>a</sup>				1989-1990				1990-1991				1991-3/2/1992				3/3/1992-6/30/93			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Enrollment	1	0	1	9	26	6	14	3	16	9	22	16	10	13	5	12	18	19	1	0
Enrollment Goals	80	40	48	72	80	40	48	72	80	40	48	72	45	40	48	72	45 <sup>b</sup>	29 <sup>b</sup>	49 <sup>b</sup>	40 <sup>b</sup>
Cumulative Enrollment	1	0	1	9	27	6	15	12	43	15	45	32	53	28	50	44	71	47	51	44
Percent Enrolled	1		2	12	34	15	48	22	54	38	94	44	118	70	104	61	157	162	104	110
# of Placentas Collected								19				35								

<sup>a</sup>= Enrollment began on 9/11/89; a total of 10 patients were enrolled during the last quarter of Year 01; <sup>b</sup>= Accrual goals were revised per Modification P30008.

## Appendix V

### $\beta_2$ Microglobulin Assay

Aliquots of serum were analyzed for  $\beta_2$ M using the microparticle enzyme immunoassay (MEIA) (IMX  $\beta_2$  microglobulin; Abbott Laboratories, Abbott Park, Illinois, USA). This method combines an antigen-antibody reaction with an enzyme rate reaction. Briefly, 150  $\mu$ l of the serum sample is diluted 10-fold with buffer solution and the anti- $\beta_2$ M antibody coated microparticles are added to the incubation well of the reaction cell.  $\beta_2$ M in serum binds to the antibody coated microparticles forming an antibody-antigen complex. The microparticles are transferred and bind irreversibly to a glass fiber matrix. The matrix is washed to remove all unbound material and anti- $\beta_2$ M alkaline phosphatase conjugate is added. Finally, the substrate 4-methylumbelliferone phosphate is added to the matrix and the fluorescent product is measured by the IMX optical assembly. The rate of generation of the fluorogenic product is proportional to the concentration of  $\beta_2$ M in the test sample. The lowest detectable level of  $\beta_2$ M by this assay is 5  $\mu$ g/l. Each time a test is performed 6 controls are included for reference ranges of low, medium and high  $\beta_2$ M levels. Ninety-five percent of specimens from healthy individuals have values  $\leq 1.9$   $\mu$ g/l in our laboratory. The coefficients of variation among repetitive samples and among different laboratories with this assay range between 6.6-7.3% and 7.3-9.2%, respectively. Specimens with values exceeding 4000  $\mu$ g/l were diluted 10 fold to quantify the results.

## APPENDIX VI PUBLICATIONS/ABSTRACTS

**Bardeguetz, A.D., Taylor, U., Apuzzio, J., Leus, C., Lister M., Denny, T., Palumbo, P., Connor, E:** Characteristics of Pregnant Women Infected with Human Immunodeficiency Virus 1: Newark Perinatal Transmission Study. Presented at the Seventh International Conference on AIDS, Florence, Italy June, 1991.

**Bardeguetz, A., Taylor, U., Stephen, R., Holland, B., Oleske, J., Shefton, D., Denny T., Leus, C., Connor, E:** Serum B2 Microglobulin Levels in Pregnant HIV Infected Women. Accepted for poster presentation at the Society for Gynecologic Investigation, March 1992 #493.

**Bardeguetz, A., Taylor U., Wesley Y., Denny T., Connor E.** HIV Infection in Childbearing Women Associated STD's Infections and Immunological Profile. Accepted for poster. Soc Perinatal Obstetricians, San Francisco, February, 1993.

**Bardeguetz, A., Palumbo, P., Wesley, Y., Holland, B., Denny, T., Connor, E.** T-Cell Subsets During Pregnancy and Postpartum. IXth International Conference on AIDS, Berlin, October, 1993.

**Bardeguetz, A., Denny, T., Palumbo, P., Wesley, Y., Oleske, J., Connor, E., Weiss, G.** A Rationale for HIV-1 Counselling and Screening Programs in the Prenatal Setting. Submitted for publication.

**Bardeguetz, A., Holland, B., Wesley, Y., Denny, T., Connor, E., Taylor, U., Netta, D., Palumbo, P., and Oleske, J.** The Impact of Substance Use and HIV-1 Infection on Perinatal Outcome Experience of the Perinatal HIV-1 Transmission Study. Submitted for publication.-

**Bardeguetz, A. Taylor U, Oleske J., Denny, T. Leus, C. Connor, E.** The Perinatal Human Immunodeficiency Virus Transmission Study - Newark - Interim Report. Society for Perinatal Obstetricians Annual Meeting [submitted].

**Bardeguetz, A. Taylor U, Connor, E., Kloser, P., and Weiss, G.** Newark Prenatal HIV Clinic. Society for Perinatal Obstetricians Annual Meeting [submitted].

**Connor, E., et al.** Enzyme Immunoassay for Detection of Human Immunodeficiency Virus-Specific Immunoglobulin A Antibodies. J Clin Microbiol 1993; 31(3): 681-684.

**Connor, E.** Advances in Early Diagnosis of Perinatal HIV Infection. JAMA 1991; 266(24): 3474-75.

**Connor, E.M., Bagarazzi, M., McSherry, G., et al.** Clinical and Laboratory Correlates of Pneumocystis Carinii Pneumonia in Children Infected with HIV. JAMA 1991; 265:1693-97.

**Connor, E., Bardeguet, A., Apuzzio, J.** The Intrapartum Management of the HIV-Infected Mother and Her infant. Clinic in Perinatol 1989; 16(4): 899-908.

**Denny, T., Jensen, B., Garcia, A., et al.** Measurement of Absolute CD4 + and CD8 + Lymphocyte Levels in a High HIV + Prevalent Population by a New Alternative Technology (abstract). Presented at Biotech 1994, Florence, Italy, April 1994.

**Denny, T., Jensen, B., Gavin, E., et al.** Determination of CD4 and CD8 Lymphocyte Subsets by a New Alternative Fluorescence Immunoassay. Clin and Diagnostic Laboratory Immunol 1995; 2(3): 330-36.

**Kovacs, A., Oleske, J.M.** Medical Management and Care of Newborns and Infants born to HIV-Seropositive Mothers. In: Pediatric AIDS: The Challenge of HIV Infection in Infants, Children and Adolescents. Wilkins & Wilkins, 1994.

**Palumbo, P., Allain JP, Delaney S., Bardeguet, A. Connor, E.** Hepatitis C in HIV-infected Pregnant Women and Children. Presented at the Seventh International Conference on AIDS, Florence, Italy June, 1991.

**Palumbo, P., Bardeguet, A., Wesley, Y., Lewis, D., Oleske, J., Connor, E.** Analysis of HIV Infection Status in Pregnant Women and Correlation with Infant Outcome. IXth International Conference on AIDS, Berlin, October, 1993.

**Taylor, U., Bardeguet, A:** Antiretroviral Therapy During Pregnancy and Postpartum. Accepted for poster presentation at the Society of Perinatal Obstetricians, February 1992.

**Taylor, U., Gascon, P., Apuzzio, J., Bardeguet, A:** HIV-Associated Immune Thrombocytopenia in Pregnancy. Accepted for poster presentation at the Society of Perinatal Obstetricians, February 1992.

**Taylor, U., Apuzzio, J., Bardeguet, A.** Cerebrospinal VDRL for detection of early neurosyphilis in HIV Infection Pregnant Women. Society of Perinatal Obstetricians Annual Meeting [submitted]

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